भारतीय मानक
Indian Standard

IS 548 (Part 3/Sec 1) : 2021

खाद्य तेल और वसा के लिए नमूनाकरण और परीक्षण की विधि

भाग 3 उन्नत उपकरणीय विधियां

अनुभाग 1 वसा अम्ल प्रोफाइल का निर्धारण

Methods of Sampling and Test for Oils and Fats

Part 3 Advanced Instrumental Methods

Section 1 Determination of fatty acid profile

ICS 67.200.10

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June 2021 Price Group 3

Oils and Oilseeds Sectional Committee, FAD 13

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Food and Agriculture Division Council.

IS 548 was first published in 1954 covering the methods of sampling, physical, chemical and qualitative tests for oils and fats. Subsequently, the standard was revised in 1964 and split into 3 parts namely, Part I Methods of sampling, physical and chemical tests; Part II Methods for purity tests; and Part III Methods of analysis of vegetable oils and fats by gas liquid chromatography (GLC) technique (first published in 1976).

To update the method for fatty acid profile given in IS 548 (Part III) and to include advanced analytical methods for the additional quality parameters, IS 548 Part III is being renamed as 'Advanced instrumental methods' and split into separate sections:

Section 1	Determination of fatty acid profile
Section 2	Determination of oxidative stability (accelerated oxidation test)
Section 3	Determination of the triacylglycerol composition of fats and oils — Determination by capillary gas chromatography
Section 4	Determination of content of polar compounds
Section 5	Determination of wax content by gas chromatography
Section 6	Determination of phosphorus content — Colorimetric method
Section 7	Determination of phosphorus content — Method using graphite furnace atomic absorption spectrometry
Section 8	Determination of phosphorus content — Method using inductively coupled plasma (ICP) optical emission spectroscopy
Section 9	Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS — Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol
Section 10	Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS — Method using slow alkaline transesterification and measurement for 2-MCPD, 3-MCPD and glycidol
Section 11	Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS — Method using acid transesterification and measurement for 2-MCPD, 3-MCPD and glycidol

This section of IS 548 (Part 3/Sec 1) prescribes method for determination of fatty acid profile for oils and fats and thus forms a necessary adjunct to the series of Indian Standard specifications for individual oils and fats. This standard IS 548 (Part 3/Sec 1) would supersede IS 548 (Part 3) : 1976 'Methods of sampling and test for oils and fats — Analysis by gas liquid chromatography'.

The composition of the Committee responsible for formulation of the standard is given in Annex A.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD OF SAMPLING AND TEST FOR OILS AND FATS

PART 3 ADVANCED INSTRUMENTAL METHODS

Section 1 Determination of fatty acid profile

1 SCOPE

This standard (Part 3/Sec 1) prescribes the method of test for determination of fatty acid profile of oils and fats.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
IS 548 (Part 1/Sec 1) : 2021	Method of sampling and test for oils and fats: Part 1 Sampling, physical and chemical tests, Section 1 Sampling
1070 : 1992	Reagent grade water — Specification (*third revision*)

3 QUALITY OF REAGENTS

Unless otherwise specified, pure AR grade chemicals and distilled water (*see* IS 1070) shall be employed in tests.

4 SAMPLING

Representative samples of the material shall be drawn as prescribed in IS 548 (Part 1/Sec 1).

5 FATTY ACID PROFILE ANALYSIS

5.1 Method A

5.1.1 *Principle* — The methyl esters are formed using boron trifloride or methanol and alkali and separated by gas – liquid chromatography using a flame ionization detector. The pattern of methyl esters can be compared with authentic oils for identification.

5.1.2 *Apparatus*

5.1.2.1 Gas liquid chromatograph with the following characteristics:

a) Injection system heated to a temperature of 20-50 °C higher than the column.
b) Oven — capable of heating the column to at least 220 °C and maintaining the temperature to within ± 1 °C. If temperature programming is to be employed, twin columns are recommended.
c) Packed column — may be glass or stainless steel. However glass is preferred as steel may decompose polyunsaturated fatty acids having more than 3 double bonds. Some successful column packings with column length, internal diameter and operating temperature are as follows:
    1) 12-15 percent ethylene glycol succinate on 100/120 mesh gas chrom P (2 m × 4 mm, at 180 °C);
    2) 2-10 percent apizon – L on 80/100 mesh Chromosorb W or Celite (2 m × 4 mm at 220 °C);
    3) 10 percent butan-1-4 diol succinate on 80/100 mesh Chromosorb W or celite (2 m × 4 mm at 175 °C);
    4) 3 percent SE – 30 on 100/120 mesh Chromosorb – G silanised (2 m × 3 mm at 190 °C) Condition the newly prepared column by disconnecting the detector and heating the column in the oven to the normal operating temperature for 16 h while running the carrier gas at a rate of 20-60 ml/min; and
    5) Detector – Flame ionization detector – capable of being heated to a temperature above that of the column.

5.1.2.2 *Syringe* — 10 µl graduated in 1/10th of a microlitre.

5.1.2.3 *Recorder* — electronic with high precision with rate of response below 1.5 s, width of paper 25 cm, paper speed 25-150 cm/hr.

5.1.2.4 Integrator or calculator for rapid and accurate calculations.

5.1.2.5 50 and 100 ml boiling flasks.

5.1.2.6 Reflux condenser.

5.1.2.7 Graduated pipette – 10 ml.

5.1.2.8 Test tubes with ground stoppers.

5.1.2.9 250 ml separating funnels.

5.1.3 *Reagents*

5.1.3.1 *Carrier gas*

Inert gas (nitrogen, helium, argon) thoroughly dried and containing less than 10 mg/kg of oxygen.

5.1.3.2 Auxillary gas hydrogen 99.9 percent minimum purity. Free from organic impurities, air or oxygen.

5.1.3.3 *Reference standards* — A mixture of methyl esters or methyl esters of oils of known purity preferably similar to the fatty matter being analysed.

5.1.3.4 *Methanolic Sodium hydroxide solution* — Approx 0.5 N. Dissolve 2 gm of sodium hydroxide in 100 ml methanol containing not more than 0.5 percent m/m water. When the solution has to be stored for considerable time, a small amount of white ppt of sodium carbonate may be formed. This has no effect on the preparation of the methyl esters.

5.1.3.5 *Methanolic solution of boron trifloride* — 12-15 percent m/m, 14 and 50 percent solutions are commercially available. The methanolic solution of boron trifloride should be stored in a refrigerator.

5.1.3.6 *Heptane-chromatographic quality*

5.1.3.7 *Redistilled petroleum ether 40 – 60 °C*

5.1.3.8 *Anhydrous sodium sulphate*

5.1.3.9 *Saturated solution of sodium chloride*

5.1.3.10 *Methyl red* — 1 gm/litre in 60 percent alcohol.

5.1.4 *Procedure*

Prepare the methyl esters of the fatty acids. The method using boron trifloride gives good results and is preferable to alternative methods which may be used when boron trifloride is not available. Because of the toxic character of boron trifloride various operations must be performed under a ventilated hood. All glass ware must be washed with water immediately after use. If the oil or fatty acids include fatty acids containing more than 2 double bonds it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few minutes. Transfer about 350 mg of clear oil to a 50 ml conical flask, add 6 ml of 0.5 N methanolic sod. hydroxide solution, 7 ml of boron trifloride solution and a boiling chip. Fit the condenser to the flask. Boil under reflux until the droplets of oil disappear (5-10 min). Add the appropriate amount of boron trifloride solution with a bulb or automatic pipette through the top of the condenser. Continue boiling for 2 min. Add 2-5 ml of heptane to the boiling mixture through the top of the condenser. Continue boiling for 1 min. Withdraw the source of heat and then remove the condenser. Add a small amount of saturated Sod. Chloride solution to the flask in order to bring the level of liquid into the neck of the flask. Transfer about 1 ml of the upper layer (heptane solution) into a test tube with a ground glass neck and add a little anhydrous sod. sulphate to remove any trace of water. This solution will contain about 5-10 percent of methyl esters and may be injected directly into the column of gas liquid chromatograph.

5.2 Method B (Alternate Method for Preparing Methyl Esters)

5.2.1 *Principle*

Methyl esters can also be prepared without the use of boron trifloride. This involves methyl esterification of the fatty acids in an alkaline medium and is suitable for neutral oils and fats with an acid value less than 2.

5.2.2 *Reagents*

5.2.2.1 Methanol containing not more than 0.5 percent water.

5.2.2.2 *Methanolic potassium hydroxide solution* — Approx 1 N. dissolve 5.6 gm potassium hydroxide in 100 ml of methanol containing not more than 0.5 percent m/m water (anhydrous methanol).

5.2.2.3 *Heptane chromatographic quality*

5.2.2.4 *Anhydrous sodium sulphate*

5.2.2.5 *Nitrogen,* containing not more than 0.5 mg/kg of oxygen.

5.2.3 *Procedure*

If the oil includes fatty acids containing more than 2 double bonds, it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few minutes. Transfer about 4 gm of clear sample oil into a 100 ml round bottomed or conical flask. Add about 40 ml of methanol, 0.5 ml of methanolic potassium hydroxide solution and a boiling chip. Fit under a reflux condenser, stir and bring to boil. The solution should become clear (5-10 min). Cool under running water and transfer the contents to a 125 ml separating funnel, rinsing the flask with 20 ml of heptane. Add about 40 ml water, shake and allow to separate. The esters pass into the upper heptane layer. Separate extract of the aqueous layer again with 20 ml heptane. Combine the two extracts and wash them with several 20 ml portions of water. Separate and dry the ester solution over anhydrous sodium sulphate. Filter through cotton wool into a 50 ml conical flask and evaporate solution to approx 20 ml on a water bath while passing a stream of nitrogen.

5.3 Determination of Fatty Acids

Programme GC to maintain column temperature of 185 °C and detector temperature at 200 °C. Inject 0.1-2 µl of 5-10 percent of heptane solution of methyl esters by piercing the septum of the inlet port. Withdraw needle and note formation of a small peak on the chart paper due to solvent making start reference point. It is possible to work with lower column temperature where the determination of acids below C_{12} is required or higher temperature when determining fatty acids above C_{20}. It is also possible to employ temperature programming to take care of both situations. Analyse reference standard mixture of known composition in the same operating conditions as those employed for the sample and measure the retention distances or retention times for the common fatty esters. Identify the peaks for the sample from the graph. If an integrator is used, obtain the figures from it.

NOTE — Fatty acids appear on the chart in increasing number of carbon atoms and increasing unsaturation. Thus, C_{16} appears before C_{18}, $C_{18:1}$ before $C_{18:2}$ and so on.

ANNEX A

(*Foreword*)

COMMITTEE COMPOSITION

Oils and Oilseeds Sectional Committee, FAD 13

Organization	*Representative(s)*
Harcourt Butler Technological University, Kanpur	Dr R. K. Trivedi (***Chairman***)
All India Food Processors' Association (AIFPA), New Delhi	Shri D. V. Malhan Dr K. L. Gaba (*Alternate*)
Cargill India Pvt Ltd, Gurugram	Mr Neeraj Kumar Singh Ms Nidhi Bhatiwada
Center for Rural Development and Technology, IIT, Delhi	Shri S. N. Naik Shri Jatindra Sahu (*Alternate*)
CONCERT Trust, Chennai	Mr K. Krishnakumar Mr M. R. Krishnan (*Alternate*)
Consumer Guidance Society of India, Mumbai	Dr Sitaram Dixit Dr M. S. Kamath (*Alternate*)
CSIR Central Food Technological Research Institute (CFTRI), Mysore	Dr R. Subramanian Dr Ajay Wamanrao Tumaney (*Alternate*)
Defence Food Research Laboratory, Mysore	Dr A. D. Semwal Dr K. Jayathilakan (*Alternate*)
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Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur (Rajasthan)	Dr Anubhuti Sharma Dr Arun Kumar (*Alternate*)
FARE Labs Pvt Ltd, Gurugram	Mr D. Mathur Dr Meenakshi Sharma (*Alternate*)
Food Safety and Standards Authority of India, New Delhi	Ms Aiman Zaidi
Harcourt Butler Technological University, Kanpur	Dr P. K. S. Yadav
Institute of Chemical Technology, Mumbai	Prof R. D. Kukarni
Indian Institute of Chemical Technology, Hyderabad	Dr Pradosh P. Chakrabarti Dr Sanjit Kanjilal (*Alternate*)
Indian Oilseeds and Produce Export Promotion Council	Mr Jitendra Bhushan Misra Mr Kishore Tanna (*Alternate*)
Indian Vanaspati Producers Association (IVPA), New Delhi	Shri S. P. Kamrah Shri S. K. Handu (*Alternate*)
Marico Ltd, Mumbai	Ms Meenu Yadav Ms Reshma Poothatta (*Alternate*)
Mustard Research and Promotion Council, New Delhi	Dr Pragya Gupta
National Institute of Nutrition, Hyderabad	Dr M. Jeyakumar Dr P. Suryanarayana (*Alternate*)
National Institute for Food Technology Enterpreneurship and Management, Sonipat	Dr Sunil Pareek
National Oilseeds and Vegetable Oils Development Board (NOVOD), Gurugram	Shri Swapan Kumar Pal Shri Devdutt (*Alternate*)

Organization	*Representative(s)*
Oil Technologists' Association of India (OTAI), Northern Zone, Delhi	Mr C. S. Joshi Dr R. P. Singh (*Alternate*)
The Solvent Extractors Association of India, Mumbai	Dr Prabodh Halde Mr Akshay Modi (*Alternate*)
VOICE, New Delhi	Shri Ashok Kanchan Shri H. Wadawa (*Alternate*)
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In Personal Capacity	Dr A. Madhavan
In Personal Capacity	Dr Jayanti Adhikari
In Personal Capacity	Prof Amit P. Pratap
BIS Directorate General	Shrimati Suneeti Toteja, Scientist 'E' and Head (FAD) [Representing Director General (*Ex-officio*)]

Member Secretary

Dr Bhawana
Scientist 'D' (FAD), BIS

Bureau of Indian Standards

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This Indian Standard has been developed from Doc No.: FAD 13 (14789).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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Published by BIS, New Delhi